1965

A Histologic Study of the Regeneration of the Inferior Alveolar Nerve

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A HISTOLOGIC STUDY OF THE REGENERATION OF THE INFERIOR ALVEOLAR NERVE

by

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A Thesis Submitted to the Faculty of the Graduate School of Loyola University in Partial Fulfillment of the Requirements for the Degree of Master of Science

June

1965

LIFE

Robert Francis Nolan was born September 10, 1936 in Chicago, Illinois. He attended elementary school and high school in Chicago being graduated from Leo High School in June 1954. From September 1954 to June 1957 he attended Loyola University, College of Arts and Sciences. In September 1957 he entered Loyola University Dental School and was graduated in June 1961 with a Doctor of Dental Surgery degree.

Upon graduation from dental school he served in the United States Air Force from August 1961 to August 1963. In September of 1963 he enrolled in the Graduate School of Loyola University to pursue a Master of Science degree in the Department of Oral Biology.

He was appointed a Research Trainee and entered the Research Training Program, sponsored by the National Institutes of Health, at Loyola University in July of 1964.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Dr. Nicholas Choukas under whose direction this thesis was undertaken. Sincere gratitude is due Dr. Harry Sicher whose example and guidance continue to inspire me.

Thanks are due Dr. Patrick Toto who with the above served on my advisory board. Dr. Robert Pollock took the photomicrographs contained in this thesis and his help is sincerely appreciated.

Finally, special gratitude is due to my wife whose patience and understanding made the completion of this work possible.

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CHAPTER I

INTRODUCTION

There is ample clinical evidence to support the fact that the inferior alveolar nerve, upon being injured either intentionally or accidentally, does regenerate. However, there is an absence of histologic investigation of the regeneration of a nerve in a bony canal and of the influence that this canal may exert on the regeneration. Due to the role that the inferior alveolar nerve plays in the proper functioning of the masticatory apparatus - its participation in proprioception of the mandible and the maintainance of proper contour and muscle tonus of the lower lip - it is pertinant to document the process histologically by which this nerve regenerates after an interruption of its continuity.

The purpose of this investigation is to provide a histologic documentation of the regeneration of a nerve in a bony canal, in particular of the regeneration of the inferior alveolar nerve, with regard to the time sequence involved and the influence that the bony canal might exert on the process.

CHAPTER II

REVIEW OF THE LITERATURE

When a peripheral nerve fiber is cut certain events follow in different parts of the neuron. The distal segment of a nerve fiber which has been removed from connection with its cell body undergoes degeneration which begins slowly, requiring days to be completed, and involves the separate parts of the nerve fiber differently (Weddel and Glees, 1941). The axis cylinder gradually breaks up and the segments are digested and absorbed by leukocytes (Cajal, 1928). If there is a myelin sheath it is gradually transformed into a chain of lipoid droplets, the larger of which may, in the early stages, also contain degenerating fragments of the axis cylinder. The neurilemmal sheath does not degenerate, but its constituent cells proliferate forming a band or tube with many nuclei evident (Cajal, 1928). The neurilemmal tube may persist for months after the disappearance of the axis cylinder, the fragments of which disappear in a few days, or the degenerating myelin sheath, parts of which may persist as droplets for six months or more. The neurilemmal tube awaits regenerating axons but

may diminish in diameter if not reached by these axons. Regenerating nerve fibers of large size may be restricted in their growth in diameter if they enter smaller neurilemmal tubes (Hammond and Hinsey, 1945).

When a nerve fiber is cut the parts of the neuron centralward to the break also show characteristic changes. The perikaryon undergoes chromatolysis and may recover if the amount of destruction is not too great, or if it is too badly damaged it may completely degenerate (Liu, 1955). If regeneration is to occur there is a latent period of approximately 7-15 days before any fibers appear in the peripheral stump (Young, 1942; Barton, 1962).

If the cell body completely degenerates, the nerve fiber between the cell body and the cut undergoes Wallerian degeneration just as does the distal segment. If, however, the neuron survives, only a small amount of destruction occurs at the distal end of the central stump of the cut nerve (Maximow and Bloom, 1957).

From both the proximal and distal stumps of the severed nerve both the Schwann cells and fibroblasts from the peri-neural connective tissue proliferate in an attempt to bridge the defect of the sectioned nerve (Shambaugh, 1963). The axis cylinders in the central stump of the nerve divide and send out sprouts from the end of the nerve which grow

into the reactive scar tissue. Many sprouting axons go astray in random directions, but some of them eventually cross the gap and enter neurilemmal tubes persisting in the distal stump (Manter and Gatz, 1963). The rate of advance of the axon tip across the scar was estimated as 0.25 mm/day by Cajal (Young, 1942). Not all of the fibers get across the defect and those that do are directed by chance into the existing pathways in the distal stump. Chance apparently determines which neurilemmal tube a regenerating nerve fiber enters (Manter and Gatz, 1963).

The method of growth of the axon has been studied carefully by Cajal (1928) in fixed, stained preparations, by Harrison (1908) and by Shambaugh (1963) in tissue cultures, and by Speidel (1935) as the normal living nerve fiber in the tadpole's tail. All such observations serve to demonstrate that the principles of the growth of young axons are the same as the principles of growth of axons regenerating after injury (Ranson and Clark, 1959). Each newly growing nerve fiber bears an ameboid tip which projects small searching pseudopodia into the interstices of the tissue through which the fiber is growing and, while the general direction of growth is determined by other circumstances, the path of the individual fiber is influenced greatly by the terrain (Ranson and Clark, 1959; Maximow and Bloom, 1957).

As each ameboid tip moves along spinning its fiber behind it, it exhibits stereotropism, that is, the growing tip finds its way along by following previously laid down blood vessels, nerve fibers, or even connective tissue bundles (Ranson and Clark, 1959). Once a few nerve fibers have traversed a region, many others may follow along these to form a nerve bundle. In the case of embryonic tissues where a certain orderliness prevails, the path of the nerve bundle will be reasonably straight, though obstacles such as blood vessels and supporting elements can cause temporary or permanent deviation. In the regrowth of a cut nerve through scar tissue the path may be quite devious.

From a consideration of these principles of growth it is easy to see that the less the scar tissue between the cut ends of a severed nerve probably the more successful will be the restoration of fibers in the distal stump. Various artificial and natural channels have been placed between the cut ends of severed nerves to direct the growth of fibers to the distal stump.

The sprouts arising from the cut end of a nerve outnumber the fibers injured and it is possible, therefore, in particularly successful anastamosis or in the regrowth in the distal segment of a nerve crushed or injured but not separated from the central stump, to have finally a

greater number of fibers distal to an injury than proximal (Hoffman, 1955; Edds, 1951).

When the sprouts of regenerating nerve fibers reach the distal segment and find their way into the neurilemmal tubes which act as conduits their rate of growth is considerably accelerated, some having been known to grow in this location several millimeters a day (Cajal, 1928). It has been reported that the regeneration of the normal nerve structure is almost complete after eight weeks (Barton, 1962). When the new fibers have reached the site of the old endings there is another delay before the restoration of function while the endings become reorganized (Edds, 1951). As soon as the growing axon reaches a motor end-plate or a sensory end-organ it proceeds to thicken as fresh axoplasm moves into it from the cell body (Shambaugh, 1963). It has been shown by Johnson (1956) that function of a severed inferior alveolar nerve was restored after 13 weeks, determined by the disappearance of parasthesia from the affected side of the face. Thus, while the neurilemma cells appear to be incapable of developing new nerve fibers by themselves in the peripheral stump, they play an important role in nerve regeneration in cooperation with the new axons from the central stump (Ranson, 1912; Cajal, 1928).

Speidel (1935) has shown that the fibers arising as

sprouts from myelinated fibers have a greater tendency to become myelinated than those arising from unmyelinated ones, indicating a responsibility of the axis cylinder for the construction of the myelin sheath. New myelin has been seen to appear in regenerating mammalian nerves as early as 22 days after the original injury (Clark and Clark, 1947).

The amount and character of the function restored following regeneration of a cut nerve will depend upon the number of regrowing fibers which reach the proper destination and perhaps upon those which reach the wrong destination. Sensory fibers will grow down the neurilemmal tubes of motor fibers but will not substitute functionally for the motor nerves (Weiss and Edds, 1945). There is inconclusive evidence that preganglionic autonomic fibers may reinnervate skeletal muscle deprived of its nerve supply (Brown and Satinsky, 1951).

It is important to note that the nerve fibers of the brain and spinal cord, which are devoid of neurilemma sheaths, are incapable of regeneration sufficient to restore function. Abortive sprouts are put out from such central neurons which grow into the scar tissue at the site of injury. The amount of this scar tissue may be diminished under experimental conditions and the growth of axonal aprouts thus encouraged (Windle and Chambers, 1950). There have been indications that partial restoration of function has occurred across

the gap in the transected spinal cord, especially in young animals (Windle, 1955).

From a review of the available literature it becomes evident that when a peripheral nerve is severed one can expect to find the distal segment of that nerve functionally regenerated in a time approximating 3 to 4 months (Johnson, 1956; Lee 1929).

Since this study is to be performed on a nerve which is contained in a bony canal, it is also necessary to report the reaction of the bone to the surgical procedure. Since bone is composed of an unyielding mineralized intercellular substance any reaction observed in the bone will necessarily have to be carried out at the surface of the bone where the bone tissue is in contact with loose or reticular connective tissue. It is from this loose or reticular connective tissue that the osteoblasts and osteoclasts necessary for any apposition or destruction of bone must develop.

The formation of new bone begins with the apposition of osteoblasts on the bony surface and a depolymerization of the fibrils and glycoproteins of the ground substance of the connective tissue on the surface of the bone. This phase produces a homogenous substance resembling hyalin and may be termed primary osteoid tissue. The primary

osteoid tissue is then transformed into a fibrillar, calcifiable intercellular substance, the secondary osteoid tissue. This entails a reconstruction of collagen molecules into the osseous fibrils that generally have a direction entirely different from that of the fibrils of the connective tissue in which osteogenesis occurs. The third phase of osteogenesis is carried out when the unsaturated side chains of the ground substance become combined with mineral salts and the apatite crystals are formed (Weinmann and Sicher, 1955).

Immature bone is markedly different in its structure and in details of histogenesis from that of mature bone. Immature bone matrix contains bundles of coarse and irregularly arranged collagen fibrils. Osteocytes are numerous but irregular in shape and arrangement. Immature bone is always arranged in trabeculae and is, therefore, always spongy bone (Weinmann and Sicher, 1955).

Mature bone contains a smaller number of osteocytes which are more regularly shaped and arranged. The fibrils are also finer which allows for a greater volume of cementing substance which is laid down in thin layers to form lamellae instead of bars or plates as in spongy bone. Most of these lamellae are arranged in cylindrical systems (Weinmann and Sicher, 1955).

CHAPTER III

MATERIALS AND METHODS

Eleven adult mongrel dogs, nine males and two females, secured from the Loyola University Medical School, were used in this experiment. They varied in age from approximately two years to five years, and all appeared to be physically sound. The weight of the animals used varied from fifteen to fifty five pounds. The dogs subsisted on a diet of Gaines' Meal supplemented occasionally with Rival dog food and water. The animals were kept in an environment of approximately 72° F.

Eight randomly selected animals underwent unilateral sectioning of the inferior alveolar nerve while in three of the animals the nerve was sectioned bilaterally. The section of the nerve was accomplished by making a mucoperiosteal flap to expose the buccal plate of the mandible between the fourth premolar and the first molar. A 558 carbide straight fissure bur was used to open into the mandibular canal and an Orban's periodontal knife was used to dissect the contents of the canal, the inferior alveolar nerve, artery, and vein. The resulting hemorrhage was controlled by replacing the mucoperiosteal flap and suturing it tightly in place. All animals were given a postoperative

intramuscular injection of 1,200,000 units of long acting Bicillin as a prophylactic measure against postoperative infection. The animals were anesthetized by the use of Sodium Nembutal, 50 mg/ml, which was administered in dosages varying according to the weight of the animals via the intraperitoneal route. All animals appeared to tolerate the procedure well and take nourishment in the same manner as before the inferior alveolar nerve was sectioned.

The animals were sacrificed at periods of 1 week, 2 weeks, 3 weeks, 4 weeks, 2 months, 4 months, and 6 months. Overdoses of Sodium Nembutal, 50 mg/ml, were employed at sacrifice causing the animals to expire due to respiratory failure.

A block section of the mandible was removed which included the original operative site. These sections extended from the distal of the second premolar to the distal of the first molar. Block sections were also taken from the opposite body of the mandible on the normal side of two of the animals which had originally undergone unilateral section of the nerve to serve as normal controls. All sections were immersed in a fixing solution of 10% buffered formalin within 5 minutes of the time the animal was sacrificed and after the cortical plates of bone were removed to facilitate fixation. The original fixing solution

was changed after five hours and the specimens were then left in the new solution for five days until thoroughly fixed. The specimens were decalcified in a solution containing equal parts of sodium citrate 50 grams, distilled water 250 cc. and formic acid 90% 125 cc. distilled water 125 cc. until decalcification was complete. The specimens were x-rayed at regular intervals to determine when they were completely decalcified, which was at approximately 14 days.

The specimen blocks were imbedded in paraffin and sections cut longtitudinally in a buccolingual plane at 10 microns. Only central sections through the inferior alveolar nerve were selected for staining.

The sections were then stained with Hematoxylin and Eosin for routine histologic examination of the nerve injury site. Adjacent sections were then stained with Luxol Fast Blue-Periodic Acid-Schiff-Hematoxylin Stain to provide a specific histologic examination of the nerve tissue.

CHAPTER IV

FINDINGS

CLINICAL FINDINGS

In all of the animals used in this project there was a small amount of swelling noticeable in the submandibular area for approximately 72 hours following the operative procedure. No animal experienced either postoperative infection or noticeable weight loss. All mucoperiosteal flaps healed well. In all cases at the time of sacrifice it was quite obvious where the original entry into the mandibular canal was made due to the fact that the bone had not regenerated sufficiently to fill in the defect created in the buccal plate of the mandible.

HISTOLOGIC FINDINGS

ONE WEEK

In the histologic sections obtained from this specimen there was an initiation of new bone formation along the surface of the mandibular canal. This activity of the endosteum was confined mainly to the bone in the area of injury to the inferior alveolar nerve and did not diminish the diameter of the mandibular canal to any great degree at

this time. The mandibular canal contained the inferior alveolar nerve with the inferior alveolar blood vessels present in an inferior relation to the nerve. These structures were separated from each other and from the bone by layers of loose connective tissue.

The inferior alveolar nerve showed a small number of nerve fibers emanating from its severed proximal stump. There was a limited amount of proliferative activity occurring in the neurilemma of both the proximal and the distal stumps of the nerve. There was evidence of myelin degeneration and large phagocytic cells were present in these areas of degeneration in both the proximal and the distal segments of the severed nerve.

TWO WEEKS

At two weeks the bone at the surface of the mandibular canal was in a much greater state of activity than previously. The endosteum showed great numbers of cells which were undergoing mitotic activity, especially in the area of injury to the inferior alveolar nerve. Associated with the activity of the endosteum there were now very large amounts of osteophytic bone and osteoid tissue present throughout the length of the mandibular canal, with a greater thickness of osteophytic bone being concentrated in the area of injury to the inferior

alveolar nerve. These conditions existed at both the inferior and superior bony surfaces of the mandibular canal.

Loose connective tissue was present between the severed segments of the inferior alveolar nerve. The inferior alveolar blood vessels were present between the nerve and the inferior border of the mandibular canal. The remaining areas of the mandibular canal contained layers of loose connective tissue interposed between the bone, blood vessels, and nerve.

A number of nerve buds were seen emanating in a random pattern from the proximal nerve stump at this time. The cells of the neurilemma, extending only a short distance proximally from the defect but the entire distance of the nerve distally from the defect, showed an increased proliferative activity. There was an increased amount of myelin degeneration present with myelin droplets and macrophages present throughout the entire length of the distal segment of the nerve, both findings existing only for a short distance proximally from the defect in the proximal portion of the nerve.

The tissues at this time appeared to be in a more reactive state to the injury inflicted upon them than they were previously.

THREE WEEKS

The bone at the surface of the mandibular canal was

still undergoing vigorous activity at this time, especially in the area of injury to the inferior alveolar nerve. There was osteophytic bone and osteoid tissue formed at the surface of the mandibular canal which extended the entire length of the specimen, the maximum activity occurring in the area of injury to the nerve. This layer of osteophytic bone had attained a greater thickness at three weeks than in earlier specimens and consequently had caused the diameter of the mandibular canal to be reduced. The endosteum was in a very vigorous state of mitotic activity. There were also osteoclasts present at the surface of the trabecular bone in the area of injury to the inferior alveolar nerve.

Loose connective tissue was present between the proximal and distal segments of the severed nerve into which nerve fibers had penetrated from the proximal segment of the nerve. The inferior alveolar blood vessels were present between the nerve and the inferior surface of the mandibular canal. There was loose connective tissue surrounding the nerve and blood vessels, although it appeared to be present in thinner layers than previously, obviously an accommodation to the formation of osteophytic bone at the surfaces of the mandibular canal.

The proximal segment of the severed nerve showed both normal myelinization and neurilemma except at the distal end of the segment. At the distal portion of the proximal segment

there was a definite decrease in the amount of myelin present as well as a definite increase in the proliferative activity of the neurilemma. The nerve buds emanating in a random pattern from the proximal segment were more numerous than at two weeks and had progressed further across the defect area.

The distal segment of the sectioned nerve showed a definite decrease in the amount of myelin present throughout the entire length of the segment. There were more myelin droplets present with phagocytic cells approximating them. A general breakdown of the neurofibers throughout the entire length of the distal segment of the nerve was seen. Along with these changes there was an increased proliferative activity of the neurilemma of the distal segment.

TWO MONTHS

The bone at the surface of the mandibular canal had reached a more stable condition at this time. The endosteum had returned to a normal physiologic state and was no longer undergoing vigorous mitotic activity. There was no osteoid tissue present on the bony surface and the osteophytic bone appeared to be taking on lamellar patterns. The inferior alveolar blood vessels were present between the nerve and the inferior surface of the mandibular canal. There were

thinner layers of connective tissue present between the bone, the blood vessels, and the nerve than were observed previously, indicating that the lumen of the mandibular canal had been narrowed by new bone formation to a greater degree than observed at three weeks.

The nerve fibers had bridged the defect area and progressed well down the distal segment of the nerve. The nerve fiber tracts seemed to follow a more disordered course across the site of injury than they did in the control specimen. The neurilemma did not show any proliferative activity. The myelin at the distal portion of the nerve was of less quantity than that seen at the proximal end.

FOUR MONTHS

At this time the bone appeared to have reached a state of stability. The endosteum showed no signs of proliferative activity and there was no osteoid tissue present along the extent of the mandibular canal. Remnants of trabecular bone were present near the site of nerve repair which served to constrict the lumen of the mandibular canal at this point, however, not to a degree sufficient enough to cause impingement upon the nerve or the blood vessels. There were thin layers of loose connective tissue between the bone, blood vessels, and nerve.

The nerve fibers at this time had bridged the surgical defect and extended the entire length of the nerve. The myelin at the distal and proximal segments of the nerve appeared to be of similar densities, indicating the remyelinization of the distal segment of the nerve. The continuity of the nerve had been reestablished.

SIX MONTHS

The bone at the surface of the mandibular canal exhibited a state of stability. There was a small area of trabecular bone present in the area of nerve repair which served to constrict the lumen of the mandibular canal to an unappreciable degree. The remainder of the bony surface was comprised of lamellar bone. The endosteum showed no proliferative activity. There were wider areas of loose connective tissue separating the bone, blood vessels, and nerve than in the previous specimens.

The nerve fibers had bridged the defect created by the operative procedure and appeared to follow a more disorderly course across the site of repair than they did in the control specimen. The density of the myelin appeared to be uniform in both the proximal and distal portions of the nerve.

The appearance of this specimen indicated that the continuity of the nerve had been structurally reestablished

and a normal histologic state of activity had been attained by the various tissue elements.

NORMAL

The bone at the surface of the mandibular canal was arranged in lamellar patterns and no trabecular bone was present. The endosteum was in a normal physiologic state of activity and showed no areas of proliferation. The inferior alveolar nerve was present with the inferior alveolar blood vessels present inferior to the nerve. There were relatively wide layers of loose connective tissue separating these structures from each other and from the bony surfaces of the mandibular canal.

The nerve fibers in this specimen followed an orderly course throughout the entire length of the nerve. The density of the myelin was homogeneous throughout the length of the nerve. The cells of the neurilemma showed no proliferative activity.

CHAPTER V

DISCUSSION

In evaluating the results of this study it is necessary to consider the reactions of the bone and the neural elements individually as well as to consider the relation that these tissue reactions have to the overall repair process. In this way it will be possible to determine what influence the bony canal may exert on the repair process of the nerve.

The nerve tissue showed evidence of progressive myelin degeneration through the first three postoperative weeks. During this time there was also proliferative activity present in the neurilemma. Two months following section of the inferior alveolar nerve there was no proliferation of the neurilemma evident and the degree of myelinization of the severed nerve was observed to be less than that of the control specimen. The severed nerves showed the presence of axonal sprouts through the first three weeks following surgical intervention. The gap between the severed sections of the nerve was found to be spanned at two months postoperatively and the nerve was found to be structurally regenerated at four months postoperatively. These findings agree with those of Cajal, Johnson, and Young.

While the nerve tissues were carrying on their repair

process the bone was undergoing appositional activity along the walls of the mandibular canal. It is felt that the apposition of bone within the mandibular canal was a response of the bone tissue to the trauma of entering the canal and to the irritation of the blood clot that resulted from the surgery, a response to be expected from any bone that has been subjected to trauma. While large amounts of osteophytic bone were observed to be forming in the first three weeks postoperatively and served to constrict the diameter of the mandibular canal, the six month postoperative specimen presented a bony wall that contained only a very small amount of osteophytic bone and a mandibular canal diameter that was relatively similar to that of the control specimen. It is felt that the surgical window in the buccal plate of the mandible was repaired only by connective tissue because the defect was not extensive enough to threaten either the strength or function of the mandible.

The correlation between the rate at which bone is apposed on the surface of the mandibular canal and the rate at which the neural elements span the gap between the retracted segments of the sectioned inferior alveolar nerve is important. It is evident (Fig. 6) that there is a considerable amount of osteophytic bone formed in the mandibular canal and that the blood clot between the nerve segments is organized (Fig. 7)

by the third postoperative week. If the apposition of osteophytic bone was to progress to such a degree that it extended into the gap between the nerve segments or if the organization of the blood clot was to progress to the formation of dense scar tissue, the axonal sprouts and the neurilemmal cells would be blocked from spanning the gap between the nerve segments since they cannot penetrate such dense tissues. Since it has been shown that this defect is spanned by two months postoperatively it must be assumed that either the bone reaches a state of stability before it intrudes into the gap between the nerve segments or that the neural elements proliferate at a faster rate than bone is apposed and dense scar tissue is formed. It is felt that the latter is the case and that the neural elements win out over the bone and scar tissue in the "competition" to repair the defect caused by the surgical procedure.

CHAPTER VI

SUMMARY

It was the purpose of this investigation to provide histologic documentation of the regeneration of a nerve in a bony canal with regard to the time sequence involved and the influence that the bony canal might exert on the process. Mongrel dogs were selected as the subjects and the inferior alveolar nerve was surgically sectioned in the area between the fourth premolar and the first molar in eleven of these animals. The animals were sacrificed at periods of 1 week, 2 weeks, 3 weeks, 4 weeks, 2 months, 4 months, and 6 months. A block section of the mandible which included the original operative site was removed. Histologic sections were prepared and stained with Hematoxylin and Eosin for routine histologic examination and with Luxol Fast Blue-Periodic Acid-Schiff-Hematoxylin to provide a specific histologic examination of the nerve tissue. It was found that the inferior alveolar nerve exhibited apparently normal structural regeneration at four months postoperatively. The bone, which undergoes an initial vigorous appositional activity, does not appear to interfere with the regeneration of the nerve and eventually returns to a stable state effecting, at most, a slightly constricted diameter of the mandibular canal.

CHAPTER VII

CONCLUSIONS

1. The inferior alveolar nerve exhibited apparently normal structural regeneration at four months postoperatively.
2. The course of the regenerated nerve fibers across the surgical area appeared to be disordered when compared to the course of the fibers in the normal control specimen.
3. The apposition of considerable amounts of osteophytic bone on the walls of the mandibular canal did not seem to occur at a fast enough rate to endanger the regeneration of the nerve.
4. The blood clot between the nerve segments did not organize into dense scar tissue fast enough to prevent the neurilemma cells and the axonal sprouts from spanning the defect.
5. The bony canal maintained the severed ends of the nerve in close approximation without the necessity of suturing the segments together.
6. The inferior alveolar nerve showed regeneration in a length of time comparable to that reported by investigators studying regeneration of other peripheral nerves.

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APPENDIX

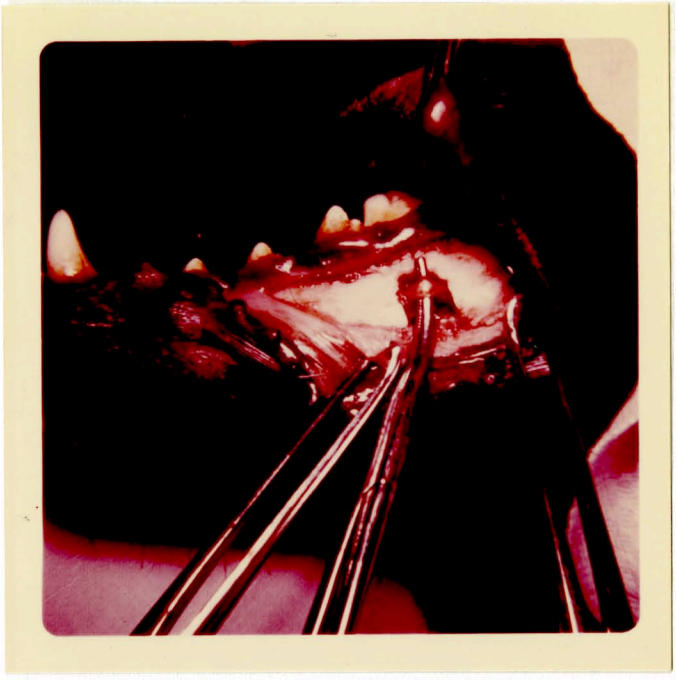

Figure 1

Demonstration of the inferior alveolar nerve through a window in the buccal plate of bone.

Figure 2

Demonstration of the surgical area after the removal of the specimen block.

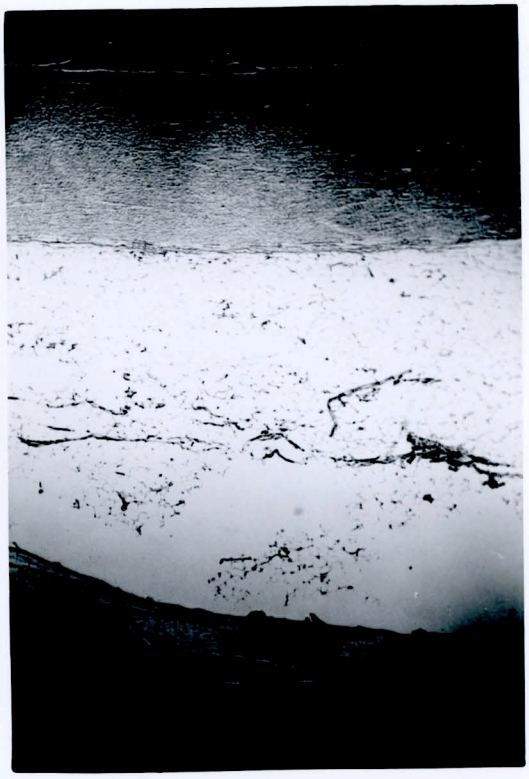

Figure 3

Control Specimen
Original magnification 25 x - H&E stain

Lamellar bone is present at the lower portion of the photograph while the nerve is present at the upper portion. The large area between these structures contains connective tissue remnants.

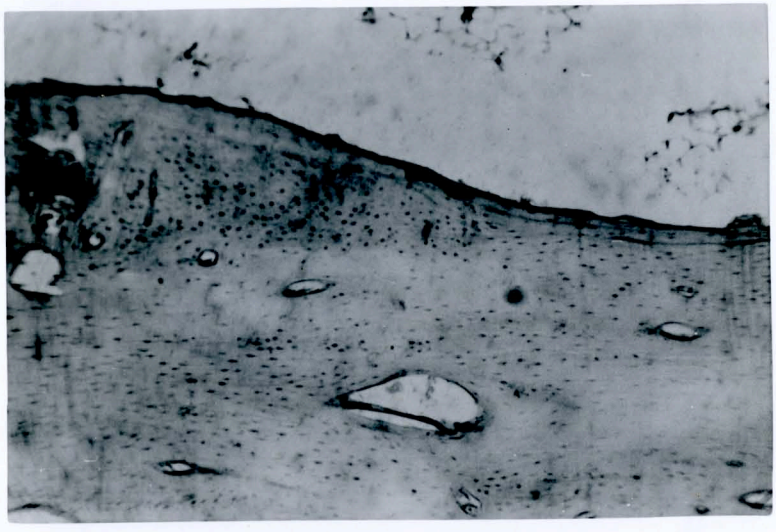

Figure 4

Control Specimen
Original magnification 60 x - H&E stain

This photograph shows the normal lamellar structure of the bone and the endosteum, which is in a normal physiologic state of activity.

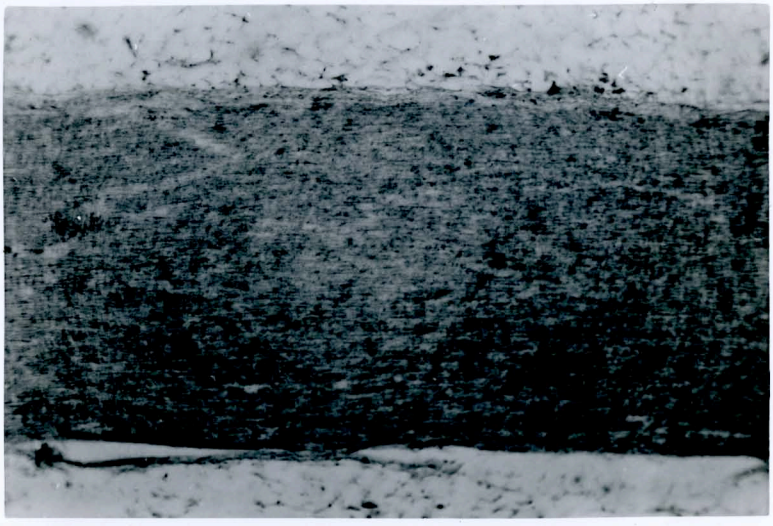

Figure 5

Control Specimen
Original magnification 60 x - H&E stain

The continuity of the inferior alveolar nerve and the orderly arrangement of the nerve fibers and neurilemmal elements are shown in this photograph. Connective tissue remnants may be seen both above and below the nerve.

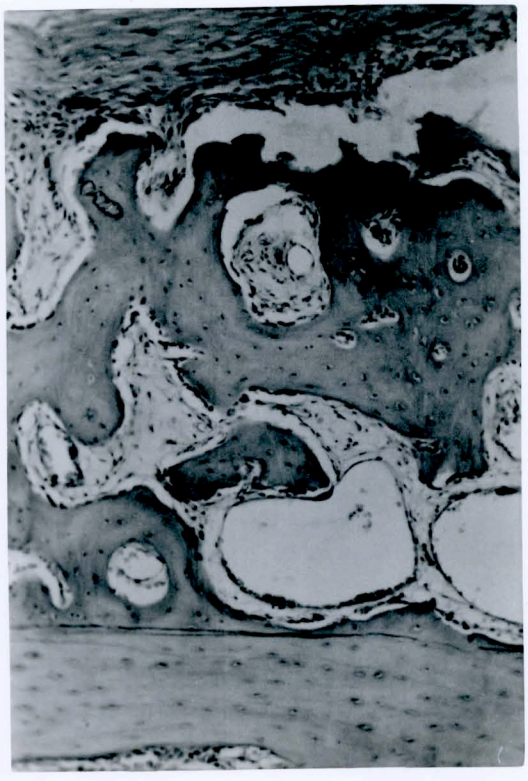

Figure 6

Three Week Specimen
Original magnification 100 x - H&E stain

Lamellar bone is present at the lower portion of the photograph with trabecular bone in the center and the very actively proliferating endosteum at the top. There is a very active process of new bone formation being carried out at this time.

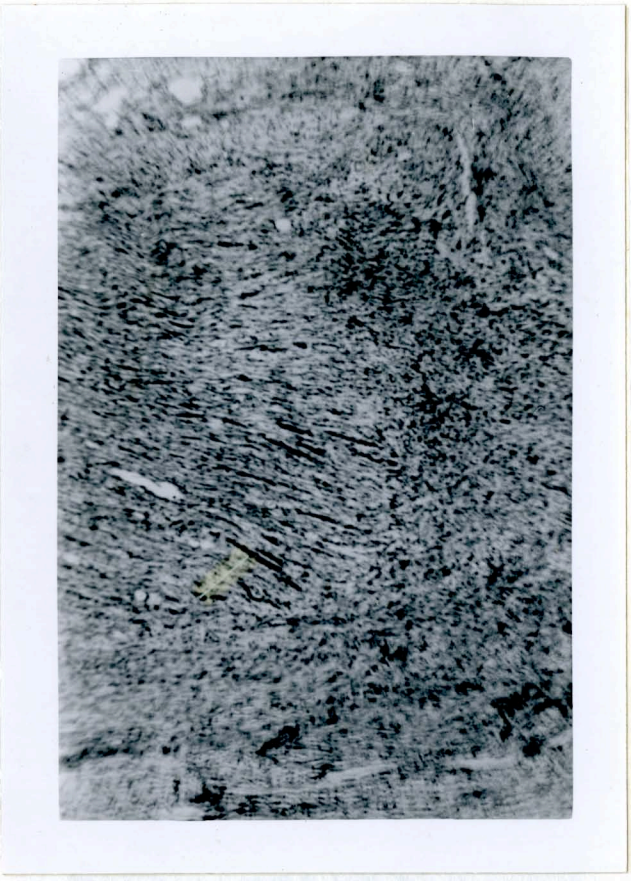

Figure 7

Three Week Specimen
Original magnification 60 x - H&E stain

This photograph shows the proliferation of neurilemmal cells (arrow) at the distal end of the proximal stump of the severed nerve. The neurofibers from the proximal stump can be seen penetrating into the loose connective tissue at the right of the photograph.

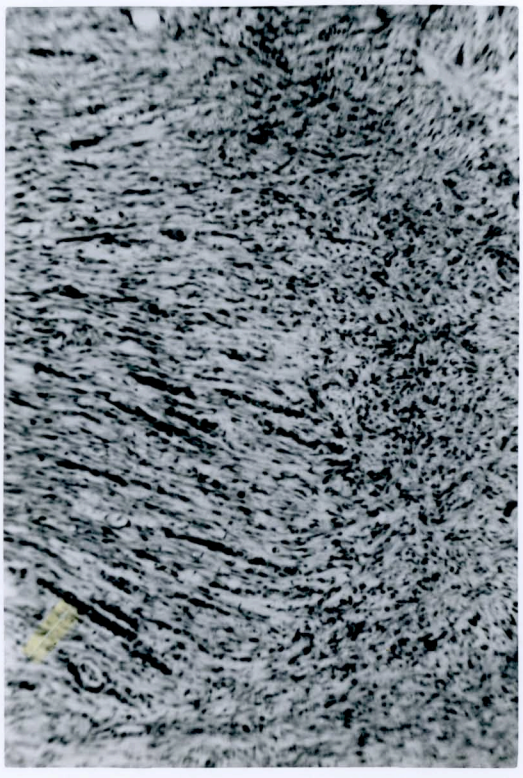

Figure 8

Three Week Specimen
Original magnification 100 x - H&E stain

The proliferation of the neurilemmal cells (arrow) is shown. The neurofibers can be seen penetrating into the loose connective tissue at the right of the photograph.

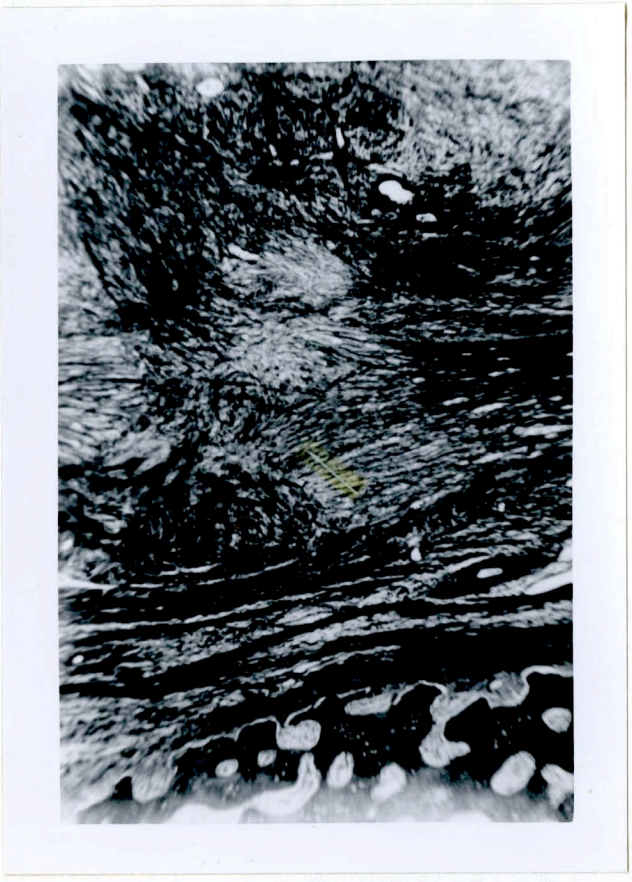

Figure 9

Three Week Specimen
Original magnification 60 x - Luxol Fast Blue stain

The penetration of the neurofibers (arrow) into the loose connective tissue can be seen in this photograph. Trabecular bone is also evident in the lower portion of the photograph.

Figure 10

Six Month Specimen
Original Magnification 25 x - H&E stain

A small amount of trabecular bone (arrow) is present in the lower right corner of the photograph. The repaired inferior alveolar nerve is present at the top of the photograph and shows a disorderly arrangement of fibers across the repair site. Immediately below the nerve is a portion of the large inferior alveolar artery.

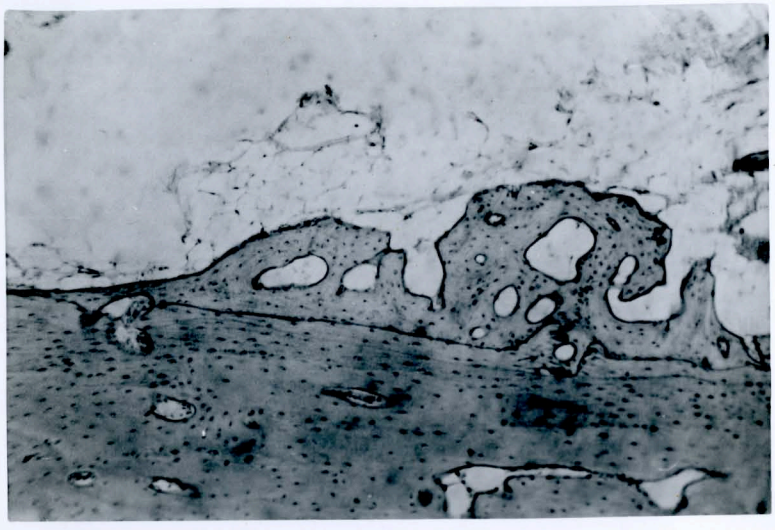

Figure 11

Six Month Specimen
Original magnification 60 x - H&E stain

There is a limited amount of trabecular bone evident in this photograph with some connective tissue remnants present immediately above the bony surface.

APPROVAL SHEET

The thesis submitted by Dr. Robert F. Nolan has been read and approved by three members of the Department of Oral Biology.

The final copies have been examined by the Director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

5/17/65
Date

Nicholas C. Cloutos DDS, MS
Signature of Adviser